

# Peak assignment in multi-capillary column–ion mobility spectrometry using comparative studies with gas chromatography–mass spectrometry for VOC analysis

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**Abstract** Over the past years, ion mobility spectrometry (IMS) as a well established method within the fields of military and security has gained more and more interest for biological and medical applications. This highly sensitive and rapid separation technique was crucially enhanced by a multi-capillary column (MCC), pre-separation for complex samples. In order to unambiguously identify compounds in a complex sample, like breath, by IMS, a reference database is mandatory. To obtain a first set of reference data, 16 selected volatile organic substances were examined by MCC-IMS and comparatively analyzed by the standard technique for breath research, thermal desorption–gas chromatography–mass spectrometry. Experimentally determined MCC and GC retention times of these 16 compounds were aligned and their relation was expressed in a mathematical function. Using this function, a prognosis of the GC retention time can be given very precisely according to a recorded MCC retention time and vice versa. Thus, unknown MCC-IMS peaks from biological samples can be assigned—after alignment via the estimated GC retention time—to analytes identified by GC/MS from equivalent accomplished data. One example of applying the peak assignment strategy to a real breath sample is shown in detail.

**Keywords** IMS · TD-GC/MS · Multi-capillary column (MCC) · Volatile organic compound (VOC) · Alignment · Breath analysis

## Introduction

Over the past few decades, ion mobility spectrometry (IMS) has become a highly interesting tool for various applications like the detection of chemical warfare agents, explosives, and drugs for military and security purpose [1, 2], for food quality and safety [3–6] or most recently for medical and biological issues [7–11]. It is a matter of common knowledge that spoilage of food by biological decomposition processes is accompanied by different characteristic smells. These putrid odors can, for instance, result from microorganisms growing on food products. Hence, detected volatile bacterial products causing such odors can prove the occurrence of microorganisms for those purposes [12, 13]. In addition, studies carried out recently suggest that the amount of different volatile biogenic amines can act as an indicator for the level of food spoilage [6, 14]. The knowledge about volatile bacterial compounds is also of great importance for medical applications and can further be used to gain information for clinical diagnosis of bacterial infections by IMS, e.g., for bacterial vaginosis [7, 8]. Interestingly enough, in the same manner as microorganisms form volatile organic compounds (VOCs), even the human cell itself is able to emit VOCs. There is evidence that the colon cancer cell line SW-480, the lung cancer cell lines CALU-1 and NCI-H2087, as well as lung tumor cells of different lung cancer patients emit VOCs in the headspace of their cell culture medium [9, 15–17]. Driven by these recent results and the long-known fact that the odor of breath can be associated with diseases [10, 18],

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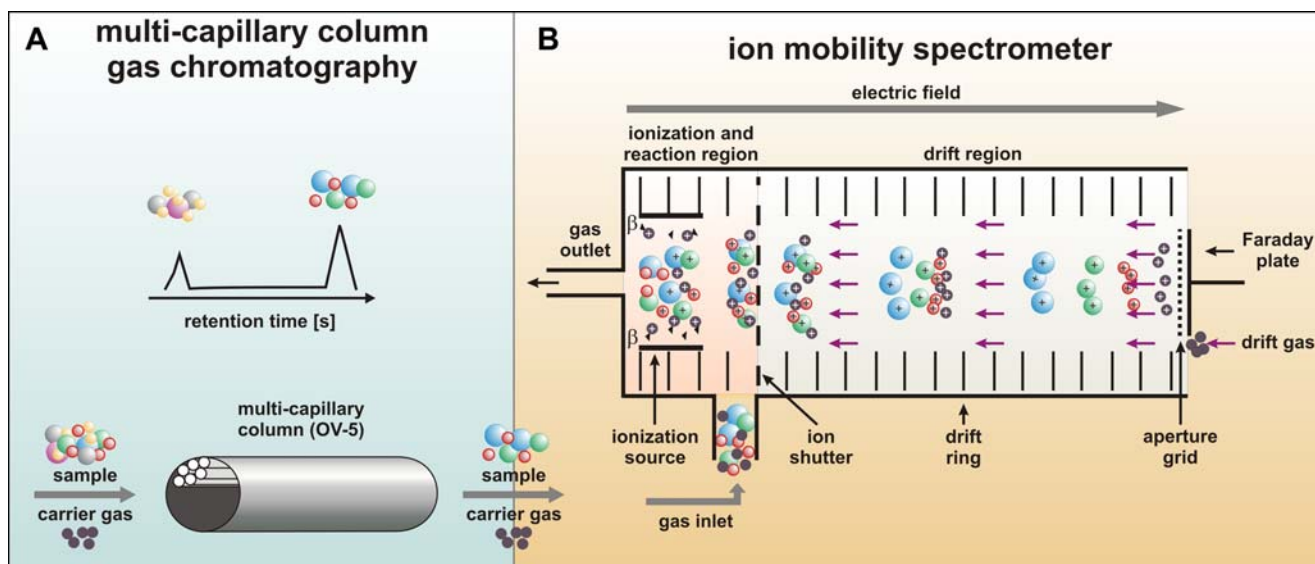
first approaches have been performed to analyze exhaled breath of patients suffering from lung cancer or sarcoidosis by gas chromatography–mass spectrometry (GC/MS) and more importantly by IMS [11, 17, 19–21].

The well-known method of IMS is based on the analysis of gas phase analytes by a rapid separation of ionized analyte molecules due to their ion mobilities in an electric field, at ambient pressure, in a particular gas (e.g. synthetic air, helium, nitrogen) as drift gas, in a drift tube [1, 2, 22]. The analyte molecules are being ionized either by UV light,  $\beta$ -radiation or by a plasma causing different sensitivities and selectivities [23, 24]. The following separation of the respective ions in the drift tube is determined by the number of collisions with neutral molecules during their acceleration in a weak electric field (Fig. 1b). Detection of such ions is realized by a Faraday plate where very small electric currents (nanoamperes to picoamperes) are generated in a time-dependent manner corresponding to the resulting mobility of the arriving ions. The time required to record a single spectrum lasts 20–50 ms, depending on the length of the drift tube and on the mobility of the particular ions. In addition to this high-speed data acquisition, IMS is furthermore characterized by its very low detection limit ranging from nanograms per liter to picograms per liter (parts per billion by volume to parts per trillion by volume) in dependence of physicochemical properties of a substance investigated [22]. Ideally, different ions reach the Faraday plate totally separated, but this cannot be realized for complex samples due to a relatively poor resolution of IMS on one hand and complex ionization reactions (charge transfer) on the other hand. Additionally, compounds with a high proton affinity are being formed preferentially. This leads to a lack of information about

compounds with a low proton affinity. Nonetheless, IMS capabilities can be enhanced even significantly firstly by coupling of IMS with mass spectrometry (MS) or secondly by combination with chromatography [24].

Firstly, IMS/MS instruments which provide unambiguous identification of ions in a mobility spectrum are commercially available since the 1970s but were mainly used for aqueous and solid samples like proteins, lipids, or alcohols and not for VOC applications. However, those instruments have predominantly been used and built by experts only in research and development laboratory [25–28]. An excellent overview about different coupling technologies of IMS with MS has been given recently [28]. Up to now, applications for VOC analysis by IMS/MS instruments have been few because of very complex equipments which are currently used in the lab only and not as portable analysis instruments.

Secondly, the combination of GC as pre-separation with IMS as second separation procedure or as detector is less complex in regard to the operating conditions and furthermore very effective [29]. Recently, a variation of a capillary GC column—the multi-capillary column (MCC)—has successfully been used in combination with IMS for analysis of biological and medical samples (Fig. 1) [11, 18, 20, 21]. Hence, this technique is a very promising tool in VOC analysis in general, including sampling [30] and powerful data interpretation tools comprising (a) smoothing and denoising to enhance the signal-to-noise ratio [31], (b) data compression mainly using wavelet methods [32–34], (c) pattern recognition [35–37], and (d) modeling of ion mobility data [38, 39]. The method, its advantages and limitations, as well as proven applications of MCC-IMS with



**Fig. 1** Scheme of an MCC-IMS. Overview screen of a multi-capillary column separation technique (A) coupled with an ion mobility spectrometer (B)

respect to rather complex and humid samples like human breath have been reviewed recently [40].

Presently, thermal desorption (TD)-GC/MS is the most common and well established method to examine and furthermore identify VOCs in various approaches like in breath analyses [10, 41–43]. In spite of its high sensitivity and reliability, GC/MS technology presents some limitations associated with its use. The disadvantages of this method especially for VOC studies are the sampling and pre-concentration steps prior to the actual measurement which might lead to contaminations and losses of analytes. Furthermore, GC/MS analyses cannot be carried out in situ, nor on-line and they are time-consuming [41, 44]. Investigations have been performed lately in order to overcome these problems using selected ion flow tube mass spectrometry (SIFT-MS) and proton transfer reaction mass spectrometry (PTR-MS). Both MS-related methods are using chemical ionization (CI) of analytes that results in only one or two characteristic ions per analyte and does not necessarily need a time-consuming pre-separation procedure. Also, a pre-concentration of samples is not needed and complex mixtures can be examined on-line in a parts per billion by volume (nanograms per liter) range regardless of water vapor, which often causes problems especially for breath samples [45]. But, besides these benefits, substances can be detected by their mass-to-charge ratio only, what makes the data evaluation still very difficult [16].

In contrast to these MS- and non-IMS-based methods (GC/MS, SIFT-MS, PTR-MS) which provide comparable sensitivities like IMS (parts per trillion by volume level (picograms per liter) [22], MCC-IMS is a low-cost device and possesses also several advantages mentioned above [24, 40]. Further advantages are due to technical properties of the MCC-IMS instrument itself such as low power supply, size, and weight that enables MCC-IMS to be used in the lab and in-field for a broad application as a very powerful tool [40].

The lack of definite identification of gas phase analytes by the suitable MCC-IMS demands the development of an analyte database containing analyte-specific retention time ( $t_R$ ) and ion mobility. One approach to identify volatile compounds obtained from originally solid samples by GC/IMS has been accomplished already. But in this case, generated gas phase analytes have previously been pyrolyzed (Py) from originally solid biological samples containing, among other things, proteins, lipids, and carbohydrates. Resulting GC peaks were identified in the following via simultaneous connection to an IMS detector and to a time-of-flight-MS by a Tee connection in the GC module [46].

In contrast to such a direct coupling and the performance of a Py, the focus of the present publication is the alignment of chromatographic data from VOCs achieved by MCC-IMS and TD-GC/MS in order to assign possible analytes to

unknown signals in complex biological MCC-IMS data. This leads to a consecutive buildup and extension of a database for VOCs detectable by the powerful tool of MCC-IMS.

The scope of this study is not the determination of detection limits but rather first steps in the establishment of an MCC-IMS database for volatile organic compounds have been made by recording and interpreting equivalently accomplished data of MCC-IMS and TD-GC/MS. The continuative objective is to enable the MCC-IMS to be used as stand-alone application for VOC analyses. Therefore, volatile substances which might be of diagnostic potential in biological and medical studies and are possibly released as VOCs from those samples have been chosen. Experiments have been performed in a similar course of action for MCC-IMS and TD-GC/MS. Finally, peak assignment strategy has been demonstrated exemplarily for a human breath sample.

## Experimental

### Chemicals and reagents

VOCs used as references were of HPLC or GC grade: acetone; hexan-2-one; heptan-2-one; octan-2-ol; (4*S*)-1-methyl-4-prop-1-en-2-ylcyclohexene ((*S*)-(-)-limonene); octan-1-ol; nonan-2-one; 5-methyl-2-prop-1-en-2-ylcyclohexan-1-ol ((-)-isopulegol); (1*R*,2*S*,5*R*)-5-methyl-2-propan-2-ylcyclohexan-1-ol ((-)-menthol); naphthalene; decanal, (5*R*)-2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one (*L*-carvone); decan-1-ol, 5-methyl-2-propan-2-ylphenol (thymol); undecan-2-ol; 2,6-diisopropylphenol (propofol); hexan-1-ol, 2-ethyl; nonanal; indole; benzaldehyde; octanal; eucalyptol; menthone; decamethylcyclopentasiloxane; anethole, and methylacetate were purchased from Sigma-Aldrich (Seelze, Germany).

### Instrumentation

#### *Multi-capillary column–ion mobility spectrometer (MCC-IMS)*

The VOC reference mixture was introduced into a sample loop (10 mL) for 4–5 s, at a flow of 100 mL/min, injected via a six-port valve and rapidly pre-separated on an OV-5 multi-capillary column (20 cm long, consisting of approx. 1,000 parallel glass capillaries, 3 mm total diameter, 43  $\mu$ m inner diameter of a single capillary; MULTICHRON, Novosibirsk, Russia), at a constant temperature of 40 °C, at a flow rate of 150 mL/min using synthetic air (scientific quality, AIR LIQUIDE Deutschland GmbH, Düsseldorf) as a carrier gas for 500 s. A breath sample (10 mL) has been taken according Westhoff et al. [21] and pre-separated as

described above. After the chromatographic separation, analytes were ionized by a radioactive ionization source ( $^{63}\text{Ni}$ , 550 MBq) and analyzed with a coupled IMS instrument according to Vautz and Baumbach [11] (custom-designed; ISAS, Dortmund, Germany) with the following parameters: synthetic air as drift gas, at a flow rate of 100 mL/min, in an electrical field of 310 V/cm, drift tube (length 12 cm, 15 mm diameter), shutter grid opening time of 300  $\mu\text{s}$ , spectra length/sample interval of 100 ms and spectra resolution of 50 kHz.

#### *Thermal desorption–gas chromatography–mass spectrometry*

The headspace of the mixture of reference compounds and 1 L of human breath were loaded onto a thermal desorption tube packed with 200 mg of Tenax<sup>TM</sup> GR (GERSTEL GmbH & Co. KG, Mühlheim, Germany) for 60 s at a flow rate of 50 mL/min by using nitrogen as a carrier gas and for 100 s at a flow rate of 600 mL/min, respectively. The desorption tubes were then placed in the thermal desorption system TDS A2 connected with TDS 3 (GERSTEL GmbH & Co. KG, Mühlheim, Germany) and VOCs were desorbed using the following temperature program: initial temperature of 25 °C was increased by 30 °C/s to 250 °C, 2.5 min isothermal at 250 °C, splitless for 2 min at a flow rate of 60 mL/min, using helium as carrier gas. Volatile analytes were then transferred at 300 °C to an integrated cooled injection system (CIS; GERSTEL GmbH & Co. KG, Mühlheim, Germany), focused at –120 °C, released onto a HP-5MS capillary column (60 m $\times$ 0.25 mm $\times$ 0.25  $\mu\text{m}$  film thickness; Agilent Technologies, Santa Clara, CA, USA) after the CIS was heated to 250 °C (by 12 °C/min) and temperature was held for 10 min. The GC/MS analysis was performed on an Agilent Technologies 7890A GC-system connected with an Agilent Technologies 5975C inert XL mass selective detector (MSD; Agilent Technologies, Santa Clara, CA, USA) at an initial oven temperature of 35 °C which was kept for 2 min, increased by 7 °C/min to 250 °C and held for 7 min. The separation was performed with helium as carrier gas at a constant flow rate of 1.0 mL/min. Electron ionization mode was set at 70 eV and the mass range of  $m/z$  33–450 was measured.

#### Sample preparation

##### *Preparation of VOC reference mixture*

Each substance (100–500  $\mu\text{L}$ ) has been filled into a 1-mL reaction vial (CS-Chromatography Service GmbH, Langerwehe) with a screw cap including a gas-permeable membrane (different thicknesses of various membranes to generate equal amounts of headspace concentrations (1–

10 ppbV) of every reference compound; CS-Chromatography Service GmbH, Langerwehe, Germany; polydimethylsiloxane, 1 mm thickness, Reichelt Chemietechnik GmbH & Co. Heidelberg) and the 16 reaction vials containing the reference substances and the three test compounds have been placed together in a 250-mL Schott flask with swiveling screw fitting (BOHLENDER GmbH, Grünsfeld, Germany) respectively. Prior to sampling, the Schott flask containing the respective samples has been incubated at least 72 h at room temperature under a constant flow with synthetic air of 100 mL/min.

#### Data analysis and alignment

MCC-IMS data are represented as a matrix of signal intensities where the  $x$ -axis indicates the inverse ion mobility in volt second per square centimeter, the  $y$ -axis indicates the retention time in seconds and the matrix values indicate signal intensities in volts [47]. Every occurring analyte causes an increased signal intensity at its individual matrix position. Detected analytes were then represented as a list of peaks described by the inverse reduced mobilities and retention times according to their local maxima in the data matrix. The identification of compounds in the obtained peaklist was performed by peak comparisons with reference data. TD-GC/MS data have been evaluated by MSD ChemStation Data Analysis Application (Agilent Technologies, Santa Clara, CA, USA) and compound mass spectra were identified by NIST version 2.0 (2005). For the generation of a function to suggest the GC retention time regarding to a given MCC retention time, the parameters of an appropriate function type were optimized accordingly to fit to a given set of experimentally determined MCC and GC retention times of training substances. The fitting between the suggested and experimentally determined GC retention times was performed by the nonlinear least squares fitting algorithm (NLSF) in Origin<sup>®</sup> 7G (OriginLab Corporation).

## Results and discussion

The combination of MCC and IMS provides fast separation and high sensitive analyses of complex systems. Consequently, it allows the monitoring of VOCs from complex samples and highly dynamic processes. In regard to assign a MCC-IMS signal to a compound, which is still very challenging, MCC-IMS and TD-GC/MS analyses of 16 selected VOCs (Table 1) have been carried out to enable data comparison under conditions used also for breath analyses. Chosen compounds are possibly present in various gas phase samples and/or could possess diagnostic potential. All 16 substances have been studied separately by MCC-IMS to obtain reliable information about the characteristic MCC



**Table 1** VOCs examined by TD-GC/MS and MCC-IMS to generate Eq. 1 for a data alignment

No.	Compound	MW (g/mol)	CAS #	GC $t_R$ (min) (temperature gradient)	MCC $t_R$ (s) (isotherm at 40°C)	GC $t_R$ (min) calculated	deviation of GC $t_R$ (min)	1/ $K_0$ proton-bound monomer	1/ $K_0$ proton-bound dimer
1	Acetone	58.08	67-64-1	4.98	2.9	4.95	0.03	n.d.	0.544
2	Hexan-2-one	100.16	591-78-6	9.95	4.9	10.11	0.16	0.581	0.640
3	Heptan-2-one	114.18	110-43-0	12.44	8.9	12.72	0.28	0.617	0.793
4	Octan-2-ol	130.23	123-96-6	15.11	19.9	15.34	0.23	0.698	0.885
5	(S)-(-)-limonene	136.24	5989-27-5	15.96	22.9	15.76	0.20	0.593	0.643
6	Octan-1-ol	130.23	111-87-5	16.76	34.9	16.96	0.20	0.717	n.d.
7	Nonan-2-one	142.24	821-55-6	17.30	38.9	17.27	0.03	0.688	0.904
8	(-)-Isopulegol	154.25	7786-67-6	18.73	53.9	18.17	0.56	0.681	0.920
9	(-)-Menthol	156.27	89-78-1	19.33	70.8	18.91	0.42	0.601	0.709
10	Naphthalene	128.17	91-20-3	19.75	62.9	18.59	1.16	0.542	n.d.
11	Decanal	156.20	112-31-2	19.89	92.8	19.63	0.26	0.771	0.992
12	L-Carvone	150.22	99-49-0	20.93	119.8	20.32	0.61	0.646	0.886
13	Decan-1-ol	158.28	112-30-1	21.15	191.7	21.56	0.41	0.781	n.d.
14	Thymol	150.22	89-83-8	21.60	225.6	21.99	0.39	0.627	n.d.
15	Undecan-2-ol	172.31	1653-30-1	21.74	241.6	22.17	0.43	0.797	n.d.
16	Propofol	178.27	2078-54-8	23.03	339.4	23.06	0.03	0.671	n.d.
17	Hexan-1-ol, 2-ethyl	130.23	104-76-7	15.92	23.9	15.88	0.04	0.695	0.883
18	Nonanal	142.24	124-19-6	17.66	42.9	17.54	0.12	0.749	0.941
19	Indole	117.15	120-72-9	21.86	186.7	21.49	0.37	0.567	n.d.

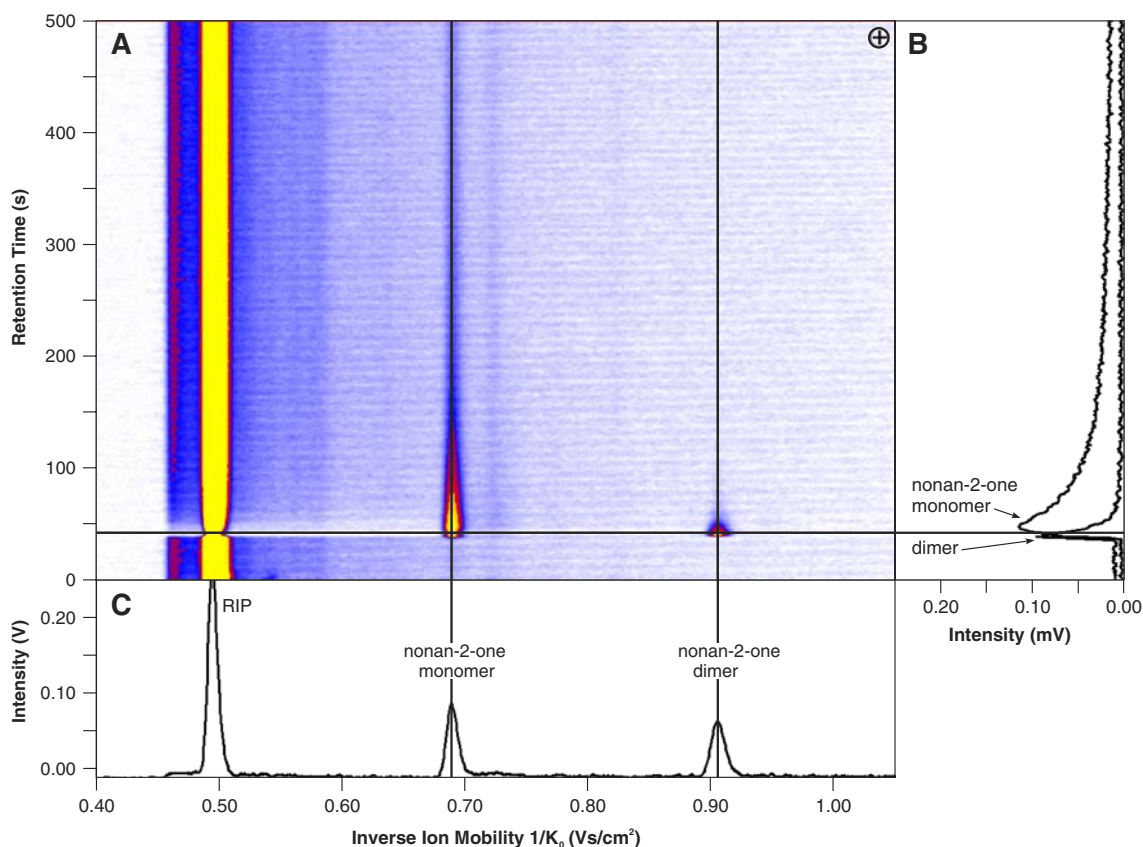
*n.d.* not detected

retention time and ion mobility which is related to ion mass, shape, and charge. Up to now, ion mobility is mostly depicted as drift time in ms, which is not consistent as it depends on ambient pressure and temperature.

Thus, the mobility has been normalized to the so-called reduced ion mobility  $K_0$  [24, 48]. In the figures, the spectra are displayed in  $1/K_0$  which is proportional to the drift time but normalized to the electric field, drift length, temperature, and pressure, and therefore represents the actual spectra resolution. Figure 2 shows exemplarily the topographic plot of positive ions of nonan-2-one. A topographic plot combines information about MCC retention time in  $s$ , inverse ion mobility  $1/K_0$  in volt second per square centimeter and signal intensity, indicated by different colors (*white* = zero, *blue* = low, *red* = medium, *yellow* = high). The retention time of nonan-2-one was determined ( $t_R = 38.9$  s) according to the MCC chromatogram (see also Table 1). Extracted ion chromatograms of the nonan-2-one protonated monomer and proton-bound dimer (Fig. 2b) reflect their formation during the ionization process that follows previous MCC separation. Proton-bound monomers, the formation of dimers or even trimers have been described for IMS previously [24]. Amongst others, the formation of such multimers has been specified to depend on physicochemical properties, the concentration of a

compound and experimental design. Quite recently, a study has been performed to specify the formation of proton-bound dimers and what still needs to be understood in detail [49]. If the concentration of nonan-2-one fell below a certain value, which was not determined here, only a proton-bound monomer peak could be observed. Furthermore, a dimerization can be anticipated for various compounds when  $^{63}\text{Ni}$  was used as ionization source [24].

In case of the occurrence of proton-bound monomer and dimer peaks, the retention times of the monomer peaks often cannot certainly be detected because of a gap in the signal intensity (Fig. 2b, Fig. 3 e.g. compound 11). However, the proton-bound dimer retention times are assigned to the appropriate monomer peaks. Additionally, the respective ion mobility spectrum (Fig. 2c) has been used to determine the characteristic  $1/K_{0,M} = 0.688$  Vs/cm<sup>2</sup> and  $1/K_{0,D} = 0.904$  Vs/cm<sup>2</sup> for the proton-bound monomeric and dimeric ion of nonan-2-one, respectively (see also Table 1). The reactant ion peak (RIP,  $1/K_{0,RIP} = 0.485$  Vs/cm<sup>2</sup>) which is always occurring when  $^{63}\text{Ni}$  is used as ionization source, can be detected at any MCC retention time, indicating detected reactant ions as products of ionized molecules of synthetic air, which are used for the ionization of analytes by charge transfer. Depending on the amount and chemical properties like the proton affinity of present analytes, the



**Fig. 2** MCC-IMS analysis of approx. 3 ppb<sub>v</sub> nonan-2-one. This compound could be detected as proton-bound monomer and dimer ion in the topographic plot (A). The overlay of MCC chromatograms from 0 to 500 s of the extracted proton-bound monomer ion at  $1/K_{0,M} = 0.688 \text{ Vs/cm}^2$  and dimer ion at  $1/K_{0,D} = 0.904 \text{ Vs/cm}^2$  of nonan-2-one

are shown in (B). The IMS spectrum reflecting the RIP, nonan-2-one proton-bound monomer and dimer at MCC  $t_R = 38.9 \text{ s}$  is displayed in (C). Peak height/intensities are indicated by different colors (white zero, blue low, red medium, yellow high)

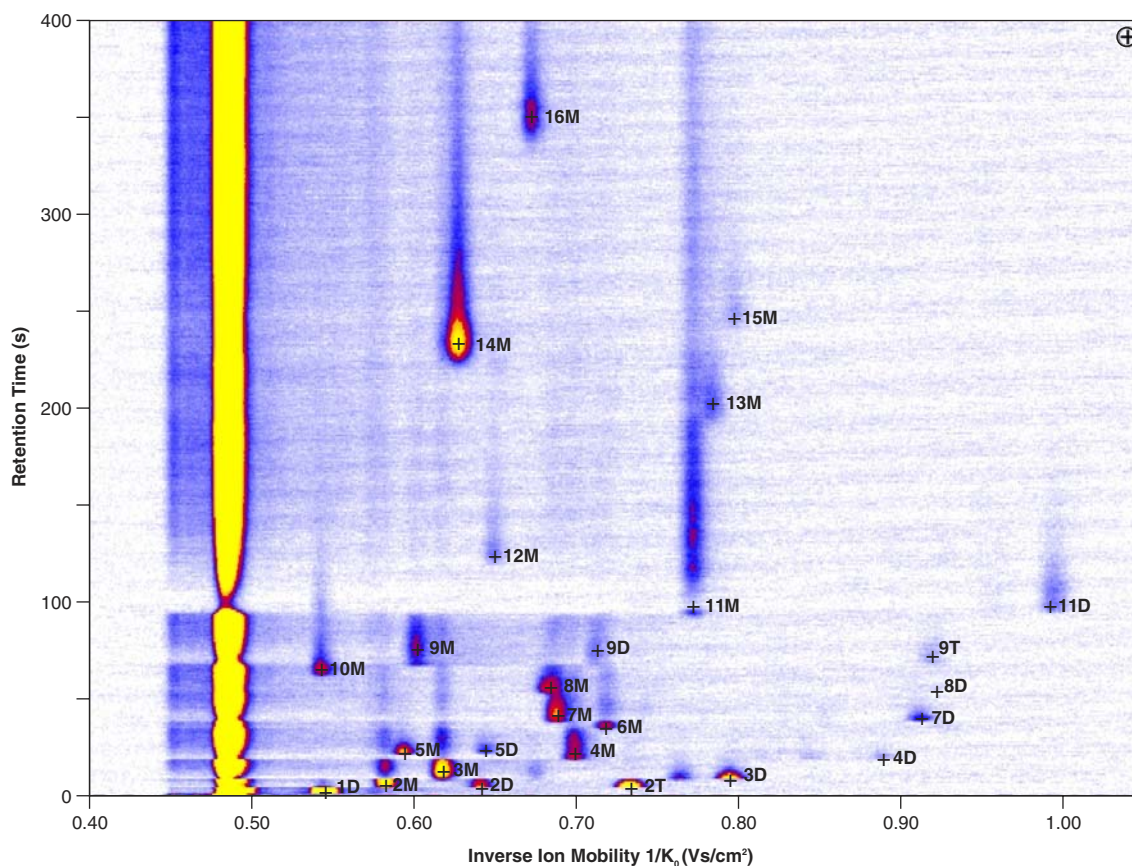
intensity of the RIP can fluctuate up to a gap of signal intensity (Figs. 2 and 3). Measured MCC retention times and ion mobilities of all 16 separately examined compounds are summarized in Table 1. In order to enable MCC-IMS to be used for compound identification in complex mixtures and to establish a reference database, those studies have to be done in the future for a huge number of volatile organic compounds.

Followed by these 16 single compound analyses, it was of further interest to determine the separation behavior of the tested substances in a mixture. As Fig. 3 reflects, all compounds could be assigned to each separately studied substance. The separation behavior of the tested chemicals was not influenced when the compounds were analyzed as a mixture by MCC-IMS. However, the formation of protonated dimer and even assumed trimer ions could be observed for several compounds, depending on their particular concentrations and on further physicochemical properties that are not completely understood or predictable, and therefore have to be investigated in later studies. Nevertheless, by experimental determination of MCC retention time and ion mobility of various volatile com-

pounds, a database for MCC-IMS data can be built for applications in the future. Examples for reference compounds and the first steps have been shown in this study.

To start with the buildup of an MCC-IMS database and to gain further information on the chromatographic separation behavior and to achieve a set of MCC and GC data in the course of developing an alignment, 16 pre-selected compounds were loaded onto a Tenax<sup>TM</sup> GR-filled TD tube and analyzed by TD-GC/MS. This compound mixture that has previously been used for the MCC-IMS experiment can on one hand be detected in various gaseous samples of different origin and furthermore covers a wide range of MCC and GC retention times. Retention times revealed by both methods were compared with the objective to build a function to suggest the GC retention time regarding to a given MCC retention time (Fig. 4).

As expected, the order of retention time for both methods was identical for most of the compounds but nevertheless differed for (–)-menthol and naphthalene. Naphthalene eluted before (–)-menthol from the MCC column (OV-5) whereas these compounds reversely eluted



**Fig. 3** Topographic plot of a MCC-IMS analysis of a mixture of 16 volatile compounds (approx. 1–10 ppb<sub>v</sub>). The mixture comprises 1 acetone, 2 hexan-2-one, 3 heptan-2-one, 4 octan-2-ol, 5 (*S*)-(-)-limonene, 6 octan-1-ol, 7 nonan-2-one, 8 (-)-isopulegol, 9 (-)-menthol, 10 naphthalene, 11 decanal, 12 L-carvone, 13 decan-1-ol,

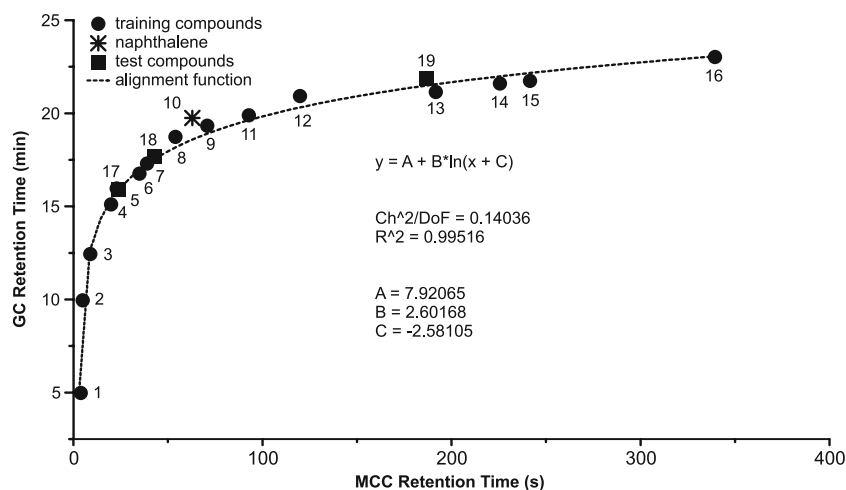
14 thymol, 15 undecan-2-ol, 16 propofol. Compounds have been detected as proton-bound monomer *M* proton-bound dimer *D* and partially as putative trimer *T*. Peak height/intensities are indicated by different colors (*white* zero, *blue* low, *red* medium, *yellow* high)

from the GC column (HP-5MS). This so-called cross-over phenomenon can occur in the course of chromatographically temperature 16 optimization processes and has been known for decades for GC analyses [50–52]. Since retention correlates with column temperature, pressure, and flow, GC and MCC temperature conditions were variegated. On one hand, the MCC isothermal temperature program (40 °C) was changed to other temperatures and on the other hand, the GC temperature gradient was changed to different isothermal conditions as well. Firstly, MCC separation of the 16 compounds was performed at column temperatures of 40 °C, 50 °C, 60 °C, and 70 °C isothermally. However, neither a temperature higher than 70 °C could be used due to material limitations of the MCC-IMS nor could a temperature gradient be accomplished concerning properties of the used multi-capillary column. A shift of retention time could be observed for all compounds from the OV-5 MCC dependent on each selected temperature, but no retention time cross-over could be detected for the tested substances.

Secondly, by changing GC separation conditions a cross-over of retention times could be ascertained for naphthalene and (-)-menthol for the chosen conditions. At 40 °C naphthalene eluted prior to (-)-menthol from the GC column which is equivalent to the retention behavior of these compounds from a MCC, whereas both substances reversely elute when 70 °C, 120 °C or a temperature gradient has been used (data not shown). Thus, the cross-over phenomenon can lead to problems in compound identification by correlation of MCC and GC data only. This finding supports the need to study every relevant volatile compound separately by MCC-IMS to establish a database and therewith unambiguously identify unknown substances by their characteristic retention time and ion mobility.

By neglecting the above-mentioned phenomenon, the elution order of substances have been compared for both chromatographic methods to establish the alignment function which is required to estimate the GC retention time.

Consequently, an appropriate function type was first appointed. The demands on such a function are that it has



**Fig. 4** Alignment of MCC and GC retention times. The  $x$ -axis describes the MCC- and the  $y$ -axis the GC retention time. 1 acetone, 2 hexan-2-one, 3 heptan-2-one, 4 octan-2-ol, 5 (*S*)-(-)-limonene, 6 octan-1-ol, 7 nonan-2-one, 8 (-)-isopulegol, 9 (-)-menthol, 11

decanal, 12 L-carvone, 13 decan-1-ol, 14 thymol, 15 undecan-2-ol, 16 propofol, 17 hexan-1-ol, 2-ethyl, 18 nonanal, 19 indole. The dotted line indicates the resulting alignment function (Eq. 1). Naphthalene (10) was excluded from the training set

to be, on the one hand, flexible enough to correctly map the retention times of the training data, and on the other hand, to preferably have a low number of parameters to avoid overfitting effects. The following logarithmic function type holds a balance between both requirements:

$${}^tR_{GCcalc}^{(x)} = A + B \cdot \ln(x + C) \quad (1)$$

The three parameters  $A$ ,  $B$ ,  $C$  (Eq. 1) were optimized using the NLSF algorithm in Origin<sup>®</sup> 7G in order to minimize the mean squared error between the suggested and experimentally determined GC retention times for the given set of training data. Because of the mentioned retention time cross-over of naphthalene, it has been excluded from the set of training data and only the 15 remaining VOCs were taken into consideration for the calculation. The resulting function parameters for this device setup are  $A=7.92065$ ,  $B=2.60168$ ,  $C=-2.58105$  with an  $R^2$  value of 0.995. These parameters have been used for the alignment calculations in Table 1 and 2 and for the plots shown in Figs. 4, 5, and 6. For other device configurations leading to a shift in retention time, e.g., by the usage of a different chromatographic column or different column temperatures, these parameters have to be recalculated according to data recorded with the respective changed setup.

Table 1 summarizes the calculated GC retention times based on experimentally determined MCC retention times according to the generated function (Eq. 1). Experimentally determined retention time and inverse reduced ion mobility ( $1/K_0$ ) of the 16 tested compounds for both chromatographic separations are given as well. The deviation between calculated and determined GC retention reflects that the used function (Eq. 1) has a relatively high precision. For

naphthalene, the calculated retention time differs from the determined retention time by 1.16 min, which is nevertheless an exception because of the retention cross-over. Apart from this compound, all other calculated values vary from 0.03 to 0.61 min for propofol and L-carvone, respectively. At the bottom of Table 1, three more compounds (17, 18, 19) are listed to serve as test substances. As Fig. 4 and Table 1 are reflecting, these data perfectly fit into the generated function (Eq. 1). Calculated and experimentally determined retention times differ between 0.04 and 0.37 min for the test compounds hexan-1-ol, 2-ethyl, nonanal, and indole.

Consequently, these differences are in the range of the deviation of training compounds. Hence, by the usage of the developed function (Eq. 1), GC retention times can be predicted with a precision of  $\pm 0.61$  min without consideration of the exception naphthalene. But even if naphthalene was taken into consideration, the preciseness with  $\pm 1.16$  min is still a very good range for the purpose of assignment of possibly present compounds in a topographical plot of MCC-IMS analyses.

The developed mathematical function (Eq. 1) was then used to visualize the match of both chromatographic retention times (Fig. 5). For a better visualization, the topographic plot ranges from 9.5 to 23.5 min (GC retention time) where every compound could be assigned unambiguously. Within the not shown range from 3.0 to 9.5 min (GC retention time) only acetone could be detected with a retention time of 4.98 min.

Since MCC-IMS is a valuable tool to analyze complex samples containing volatile compounds as shown in Fig. 3, a breath sample has been investigated by MCC-IMS and TD-GC/MS for data evaluation. With respect to assign detected MCC-IMS peaks to compounds previously used as



**Table 2** Additional VOCs examined by TD-GC/MS and MCC-IMS in a breath sample

No.	Compound	MW (g/mol)	CAS #	GC <sub>T<sub>R</sub></sub> (min)/(temperature gradient)	MCC <sub>T<sub>R</sub></sub> (s) (isotherm at 40°C)	GC <sub>T<sub>R</sub></sub> (min) calculated	deviation of GC <sub>T<sub>R</sub></sub> (min)	1/K <sub>0</sub> proton-bound monomer	1/K <sub>0</sub> proton-bound dimer
20	Benzaldehyde	106.10	100-52-7	14.34	12.4	13.88	0.46	0.561	n.d.
21	Octanal	128.21	124-13-0	15.24	19.0	15.20	0.04	0.708	n.d.
22	Eucalyptol	154.25	470-82-6	16.09	19.6	15.29	0.80	0.644	n.d.
23	Menthone	154.25	10458-14-7	18.92	50.8	18.01	0.91	0.661	0.893
24	Decamethylcyclopentasiloxane	370.78	541-02-6	18.51	76.0	19.10	0.59	0.876	n.d.
25	Anethole	148.22	104-46-1	21.60	161.1	21.10	0.50	0.593	n.d.
26	Methylacetate	198.31	89-48-5	21.75	201.9	21.70	0.05	0.599	n.d.

n.d. not detected

training and test compounds, Eq. 1 and TD-GC/MS data has been applied. More than 20 peaks have been detected and 12 VOCs have been assigned in this breath sample (Fig. 6). Five of them could be assigned to training or test substances namely (*S*)-(-)-limonene, (-)-menthol, decanal, hexanon-1-ol, 2-ethyl, and nonanal. Further on, seven additional compounds could be predicted by the described procedure using TD-GC/MS data. Subsequently, these predictions have been confirmed by separate MCC-IMS analyses of corresponding reference compounds (see Table 2).

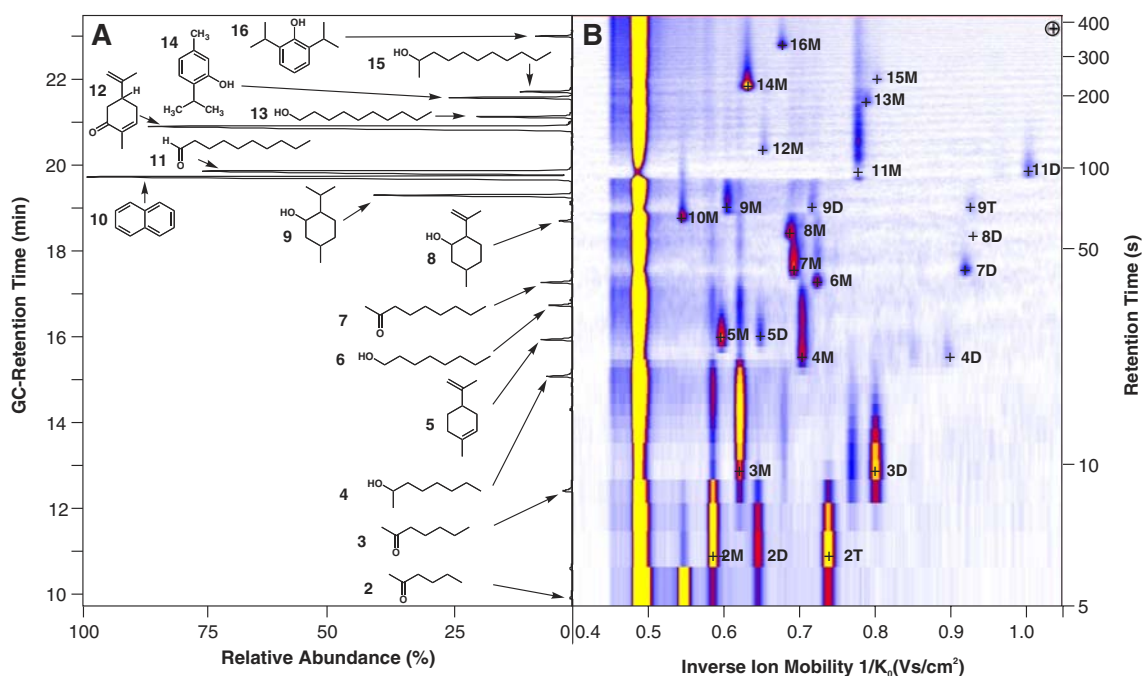
Moreover, a co-elution has been determined for (*S*)-(-)-limonene (5D) and eucalyptol (22 M) due to a high concentration of both compounds in the breath sample (Fig. 6). This separation behavior became apparent also for the separation of nonan-2-one (7 M) and (-)-isopulegol (8 M) in previously obtained data, e.g., Fig. 5. However, nonan-2-one and (-)-isopulegol are still partly separated in this sample due to a moderate examined concentration. Therefore, co-eluting compounds can be separated completely by chosen MCC-IMS conditions if their concentrations were small enough.

Volatile compounds which have been predicted to cause a MCC-IMS peak by the evolved equation (Eq. 1) and additionally obtained TD-GC/MS data are depicted in Table 2. All seven predicted substances have been analyzed separately for the determination of respective retention times and ion mobilities, and could be assigned unambiguously to the MCC-IMS peaks from the examined breath sample. For these substances, the deviation between calculated GC retention time and experimentally determined retention time was higher than for the training compounds (Table 1). Nevertheless, the highest deviation was 0.91 min which is still an acceptable range. Again a retention cross-over could be observed for menthone and decamethylcyclopentasiloxane in both chromatographic systems.

The compounds determined in the breath sample with high signal intensities like menthone or eucalyptol originate partly from oral hygiene products. Endogenous metabolites carrying information about the state of health of a person might be present at lower concentrations and also depend on the properties/quality of exhaled breath.

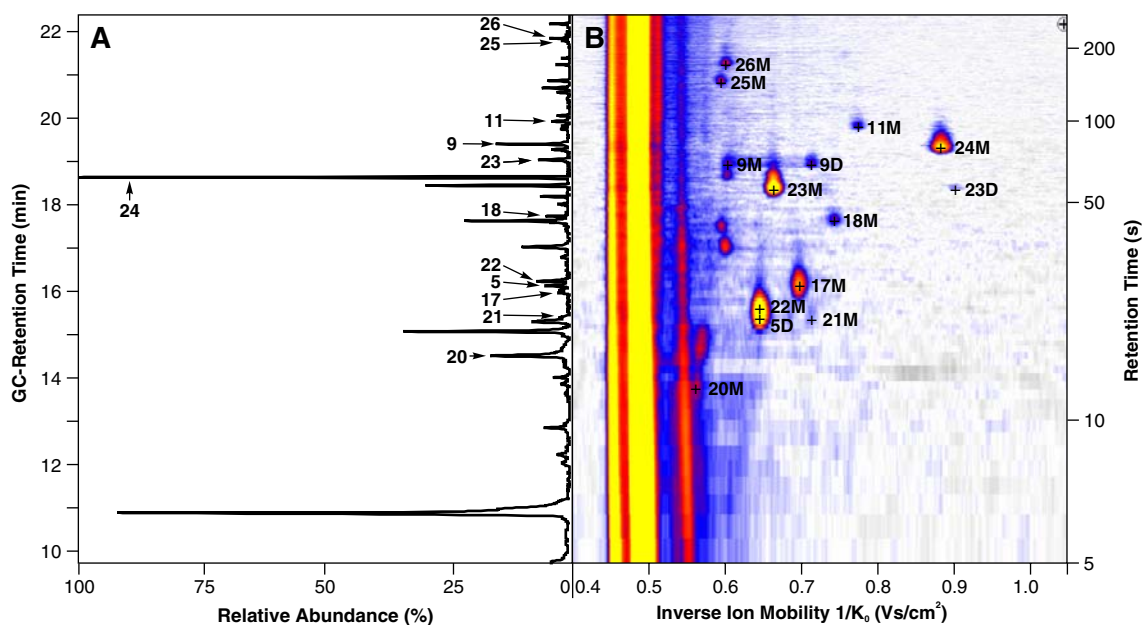
## Conclusions

It was demonstrated how analytes causing unknown MCC-IMS signals can be predicted and assigned by equivalent gained TD-GC/MS data. Therefore, an alignment of MCC-IMS and TD-GC/MS data has been performed by generating a mathematical function which describes the relation of the retention times of both different gas chromatographic methods. In order to build a training data set for the generation of the mathematical function, 16 volatile com-



**Fig. 5** Alignment of TD-GC/MS and converted MCC-IMS data of 16 volatile organic compounds using Eq. 1. **A** Shows the chromatographic plot of the GC separation from 9.5 to 23.5 min. **B** Shows the topographic plot of the MCC-IMS analysis from approx. 5 to 447 s. The mixture comprises 1 acetone, 2 hexan-2-one, 3 heptan-2-one, 4 octan-2-ol, 5 (*S*)-(-)-limonene, 6 octan-1-ol, 7 nonan-2-one, 8 (-)

isopulegol, 9 (-)-menthol, 10 naphthalene, 11 decanal, 12 L-carvone, 13 decan-1-ol, 14 thymol, 15 undecan-2-ol, 16 propofol. Compounds have been detected as protonated monomer *M*, proton-bound dimer *D* and partially as putative trimer *T*. Peak height/intensities are indicated by different colors (*white zero, blue low, red medium, yellow high*)



**Fig. 6** Alignment of TD-GC/MS and converted MCC-IMS data of volatile organic compounds from a breath sample using Eq. 1 as an example for VOC analyses. **A** shows the chromatographic plot of the GC separation from 9.5 to 23.5 min. **B** shows the topographic plot of the MCC-IMS analysis from approx. 5 to 447 s. Several peaks could be assigned 5 (*S*)-(-)-limonene, 9 (-)-menthol, 11 decanal, 17

hexanon-1-ol, 2-ethyl, 18 nonanal, 20 benzaldehyde, 21 octanal, 22 eucalyptol, 23 menthone, 24 decamethylcyclopentasiloxane, 25 anethole, and 26 methylacetate. Compounds have been detected as proton-bound monomer *M* and dimer *D*. Peak height/intensities are indicated by different colors (*white zero, blue low, red medium, yellow high*)

pounds were examined and data of 15 substances have been taken into consideration. Naphthalene has been excluded due to its different retention behavior in both chromatographic systems. The applicability and preciseness of this function has been validated by three additionally examined test substances (hexan-1-ol, 2-ethyl, nonanal, and indole). Hence, GC retention times could be predicted relatively precisely based on given MCC retention times. But, even if the elution order was changed by an occurring retention time cross-over for both chromatographic methods (for menthol and naphthalene), the quality of compound prediction was still very good. Based on the fact of possibly occurring cross-over phenomena and to further enhance the preciseness of the function, examinations of other compounds are necessary by MCC-IMS and TD-GC/MS. All these studies could then lead to establish a MCC-IMS database for volatile compounds to be used for a broad range of application like in biology or medicine.

To show the applicability of the evolved equation, a breath sample has been studied as an example for volatile samples, which are still challenging to examine. Five training compounds and seven further compounds could be assigned to unknown MCC-IMS peaks by using the equation and TD-GC/MS data.

All these compounds are present at high concentrations in breath partly derived from oral hygiene products. Further on, more unknown MCC-IMS peaks have to be assigned. Several of those compounds might be of endogenous origin and at lower levels as recently assigned hygiene products. These studies show, for the first time, how unknown MCC-IMS signals can be assigned very efficiently, leading to a stand-alone tool especially for VOC studies in the future.

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